



Nonprovisional Patent Application

17355CIP3 (BOT)

Steward, L. E. et al., Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins

SEQUENCE LISTING

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Aoki, Kei Roger

<120> Clostridial Neurotoxin Compositions and  
Modified Clostridial Neurotoxins

<130> 17355CIP3 (BOT)

<140> US 10/757,077

<141> 2004-01-14

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<150> US 10/163,106

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<160> 83

<170> FastSEQ for Windows Version 4.0

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<213> Clostridium botulinum serotype A

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<213> Rattus norvegicus

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Glu Glu Lys Arg Ala Ile Leu

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5

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<212> PRT

<213> Rattus norvegicus

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Glu Glu Lys Met Ala Ile Leu  
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&lt;210&gt; 4

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Rattus norvegicus

&lt;400&gt; 4

Ser Glu Arg Asp Val Leu Leu  
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&lt;210&gt; 5

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Rattus norvegicus

&lt;400&gt; 5

Val Asp Thr Gln Val Leu Leu  
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&lt;210&gt; 6

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 6

Ala Glu Val Gln Ala Leu Leu  
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&lt;210&gt; 7

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Xenopus laevis

&lt;400&gt; 7

Ser Asp Lys Gln Asn Leu Leu  
1 5

&lt;210&gt; 8

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Gallus gallus

&lt;400&gt; 8

Ser Asp Arg Gln Asn Leu Ile  
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&lt;400&gt; 14

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&lt;210&gt; 15

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 15

Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp  
1 5 10

&lt;210&gt; 16

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 16

Met Tyr Lys Asp  
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&lt;210&gt; 17

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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&lt;222&gt; (1)...(7)

&lt;223&gt; Consensus sequence for Leucine-based motif.

&lt;221&gt; VARIANT

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&lt;222&gt; (3)...(5)

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&lt;400&gt; 17

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&lt;210&gt; 18

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

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<213> Artificial Sequence

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<221> VARIANT  
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Tyr Xaa Xaa Xaa  
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<210> 25  
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<213> Clostridium botulinum sertotype A

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Lys Ala Phe Lys  
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<210> 26

<211> 6  
 <212> PRT  
 <213> Clostridium botulinum sertotype A

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 Phe Asp Lys Leu Tyr Lys  
 1 5

<210> 27  
 <211> 8  
 <212> PRT  
 <213> Clostridium botulinum serotype A

<400> 27  
 Pro Phe Val Asn Lys Gln Phe Asn  
 1 5

<210> 28  
 <211> 22  
 <212> PRT  
 <213> Clostridium botulinum sertotype A

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 1 5 10 15  
 Gly Ile Ile Thr Ser Lys  
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<210> 29  
 <211> 438  
 <212> PRT  
 <213> Clostridium botulinum sertotype A

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 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85 90 95  
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100 105 110  
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
 115 120 125  
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
 130 135 140



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Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145          150          155          160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
          165          170          175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
          180          185          190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
          195          200          205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
          210          215          220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225          230          235          240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
          245          250          255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
          260          265          270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
          275          280          285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
          290          295          300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305          310          315          320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
          325          330          335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
          340          345          350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
          355          360          365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
          370          375          380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385          390          395          400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
          405          410          415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
          420          425          430
Gly Ile Ile Thr Ser Lys
          435

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&lt;210&gt; 30

&lt;211&gt; 441

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum sertotype B

&lt;400&gt; 30

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Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1          5          10          15
Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
          20          25          30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
          35          40          45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
          50          55          60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn

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65		70		75		80									
Thr	Asn	Asp	Lys	Lys	Asn	Ile	Phe	Leu	Gln	Thr	Met	Ile	Lys	Leu	Phe
			85						90					95	
Asn	Arg	Ile	Lys	Ser	Lys	Pro	Leu	Gly	Glu	Lys	Leu	Leu	Glu	Met	Ile
			100						105					110	
Ile	Asn	Gly	Ile	Pro	Tyr	Leu	Gly	Asp	Arg	Arg	Val	Pro	Leu	Glu	Glu
		115					120						125		
Phe	Asn	Thr	Asn	Ile	Ala	Ser	Val	Thr	Val	Asn	Lys	Leu	Ile	Ser	Asn
		130				135					140				
Pro	Gly	Glu	Val	Glu	Arg	Lys	Lys	Gly	Ile	Phe	Ala	Asn	Leu	Ile	Ile
145					150					155					160
Phe	Gly	Pro	Gly	Pro	Val	Leu	Asn	Glu	Asn	Glu	Thr	Ile	Asp	Ile	Gly
				165					170					175	
Ile	Gln	Asn	His	Phe	Ala	Ser	Arg	Glu	Gly	Phe	Gly	Gly	Ile	Met	Gln
			180					185					190		
Met	Lys	Phe	Cys	Pro	Glu	Tyr	Val	Ser	Val	Phe	Asn	Asn	Val	Gln	Glu
		195					200					205			
Asn	Lys	Gly	Ala	Ser	Ile	Phe	Asn	Arg	Arg	Gly	Tyr	Phe	Ser	Asp	Pro
		210				215					220				
Ala	Leu	Ile	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His	Gly	Leu	Tyr
225					230					235					240
Gly	Ile	Lys	Val	Asp	Asp	Leu	Pro	Ile	Val	Pro	Asn	Glu	Lys	Lys	Phe
				245					250					255	
Phe	Met	Gln	Ser	Thr	Asp	Ala	Ile	Gln	Ala	Glu	Glu	Leu	Tyr	Thr	Phe
			260					265						270	
Gly	Gly	Gln	Asp	Pro	Ser	Ile	Ile	Thr	Pro	Ser	Thr	Asp	Lys	Ser	Ile
		275					280					285			
Tyr	Asp	Lys	Val	Leu	Gln	Asn	Phe	Arg	Gly	Ile	Val	Asp	Arg	Leu	Asn
		290				295				300					
Lys	Val	Leu	Val	Cys	Ile	Ser	Asp	Pro	Asn	Ile	Asn	Ile	Asn	Ile	Tyr
305					310					315					320
Lys	Asn	Lys	Phe	Lys	Asp	Lys	Tyr	Lys	Phe	Val	Glu	Asp	Ser	Glu	Gly
			325						330					335	
Lys	Tyr	Ser	Ile	Asp	Val	Glu	Ser	Phe	Asp	Lys	Leu	Tyr	Lys	Ser	Leu
			340					345					350		
Met	Phe	Gly	Phe	Thr	Glu	Thr	Asn	Ile	Ala	Glu	Asn	Tyr	Lys	Ile	Lys
		355					360					365			
Thr	Arg	Ala	Ser	Tyr	Phe	Ser	Asp	Ser	Leu	Pro	Pro	Val	Lys	Ile	Lys
		370				375					380				
Asn	Leu	Leu	Asp	Asn	Glu	Ile	Tyr	Thr	Ile	Glu	Glu	Gly	Phe	Asn	Ile
385					390					395					400
Ser	Asp	Lys	Asp	Met	Glu	Lys	Glu	Tyr	Arg	Gly	Gln	Asn	Lys	Ala	Ile
				405					410					415	
Asn	Lys	Gln	Ala	Tyr	Glu	Glu	Ile	Ser	Lys	Glu	His	Leu	Ala	Val	Tyr
			420				425						430		
Lys	Ile	Gln	Met	Cys	Lys	Ser	Val	Lys							
		435					440								

&lt;210&gt; 31

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 31

Tyr Ile Lys Ile

1

&lt;210&gt; 32

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 32

Tyr Asp Ser Thr

1

&lt;210&gt; 33

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 33

Tyr Gly Ser Thr

1

&lt;210&gt; 34

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 34

Tyr Asn Lys Phe

1

&lt;210&gt; 35

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 35

Tyr Met Lys Asn

1

&lt;210&gt; 36

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;400&gt; 36

Tyr Leu Asn Phe

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&lt;210&gt; 37

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<212> PRT  
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<400> 37  
Tyr Asp Gly Phe  
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<210> 38  
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Tyr Lys Leu Leu  
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<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
1 5 10 15  
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met  
20 25 30

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Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln  
1 5 10 15  
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Phe Thr  
20 25 30  
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr  
35 40 45  
Ser Lys  
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<210> 41  
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 Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr  
 20 25 30

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 35 40 45  
 Val Lys  
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 20 25 30  
 Leu Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr  
 35 40 45  
 Asn Lys  
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 20 25 30

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 Thr Lys  
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 Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr Gly Arg Gly Leu Val  
           20                          25                          30  
 Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val Ser Val Lys Gly Ile  
           35                          40                          45  
 Arg Lys  
       50

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<400> 49

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1				5				10						15	
Asp	Thr	Ile	Leu	Tyr	Met	Gln	Ile	Pro	Tyr	Glu	Glu	Lys	Ser		
		20						25					30		

&lt;210&gt; 50

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype F

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(50)

&lt;223&gt; Carboxyl terminal 50 amino acids of light chain

&lt;400&gt; 50

Thr	Val	Ser	Glu	Gly	Phe	Asn	Ile	Gly	Asn	Leu	Ala	Val	Asn	Asn	Arg
1				5				10						15	
Gly	Gln	Ser	Ile	Lys	Leu	Asn	Pro	Lys	Ile	Ile	Asp	Ser	Ile	Pro	Asp
		20						25					30		
Lys	Gly	Leu	Val	Glu	Lys	Ile	Val	Lys	Phe	Cys	Lys	Ser	Val	Ile	Pro
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Arg	Lys														
	50														

&lt;210&gt; 51

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype G

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&lt;222&gt; (1)...(30)

&lt;223&gt; Amino terminal 30 amino acids of light chain

&lt;400&gt; 51

Met	Pro	Val	Asn	Ile	Lys	Asn	Phe	Asn	Tyr	Asn	Asp	Pro	Ile	Asn	Asn
1				5				10						15	
Asp	Asp	Ile	Ile	Met	Met	Glu	Pro	Phe	Asn	Asp	Pro	Gly	Pro		
		20						25					30		

&lt;210&gt; 52

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype G

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(50)

&lt;223&gt; Carboxyl terminal 50 amino acids of light chain

&lt;400&gt; 52



Gln Asn Glu Gly Phe Asn Ile Ala Ser Lys Asn Leu Lys Thr Glu Phe  
 1 5 10 15  
 Asn Gly Gln Asn Lys Ala Val Asn Lys Glu Ala Tyr Glu Glu Ile Ser  
 20 25 30  
 Leu Glu His Leu Val Ile Tyr Arg Ile Ala Met Cys Lys Pro Val Met  
 35 40 45  
 Tyr Lys  
 50

<210> 53  
 <211> 30  
 <212> PRT  
 <213> Clostridium botulinum serotype A

<220>  
 <221> DOMAIN  
 <222> (1)...(30)  
 <223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
 <222> (4)...(4)  
 <223> Alanine substitution

<400> 53  
 Met Pro Phe Ala Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met  
 20 25 30

<210> 54  
 <211> 50  
 <212> PRT  
 <213> Clostridium botulinum serotype A

<220>  
 <221> DOMAIN  
 <222> (1)...(50)  
 <223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
 <222> (25)...(25)  
 <223> Arginine substitution

<400> 54  
 Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln  
 1 5 10 15  
 Asn Thr Glu Ile Asn Asn Met Asn Arg Thr Lys Leu Lys Asn Phe Thr  
 20 25 30  
 Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr  
 35 40 45  
 Ser Lys  
 50

<210> 55  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype A

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (10)...(10)  
<223> Lysine substitution

<400> 55  
Met Pro Phe Val Asn Lys Gln Phe Asn Lys Lys Asp Pro Val Asn Gly  
1 5 10 15  
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met  
20 25 30

<210> 56  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype A

<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (31)...(31)  
<223> Alanine substitution

<221> VARIANT  
<222> (32)...(32)  
<223> Alanine substitution

<400> 56  
Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn Gly Gln  
1 5 10 15  
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Ala Ala  
20 25 30  
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr  
35 40 45  
Ser Lys  
50

<210> 57  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype A

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (21)...(21)  
<223> Arginine substitution

<400> 57  
Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
1 5 10 15  
Val Asp Ile Ala Arg Ile Lys Ile Pro Asn Ala Gly Gln Met  
20 25 30

<210> 58  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype A

<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (13)...(13)  
<223> Histidine substitution

<400> 58  
Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn His Asn Gly Gln  
1 5 10 15  
Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu Lys Asn Phe Thr  
20 25 30  
Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg Gly Ile Ile Thr  
35 40 45  
Ser Lys  
50

<210> 59  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype A

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (7)...(7)  
<223> Histidine substitution

&lt;400&gt; 59

Met	Pro	Phe	Val	Asn	Lys	His	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn	Gly
1				5				10					15		
Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met		
		20					25						30		

&lt;210&gt; 60

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype A

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(50)

&lt;223&gt; Carboxyl terminal 50 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (43)...(43)

&lt;223&gt; Alanine substitution

&lt;400&gt; 60

Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln
1				5				10					15		
Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr
		20					25					30			
Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Ala	Arg	Gly	Ile	Ile	Thr
		35					40					45			
Ser	Lys														
	50														

&lt;210&gt; 61

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype B

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(30)

&lt;223&gt; Amino terminal 30 amino acids of light chain

&lt;221&gt; CONFLICT

&lt;222&gt; (3)...(3)

&lt;223&gt; Alanine substitution

&lt;400&gt; 61

Met	Pro	Ala	Thr	Ile	Asn	Asn	Phe	Asn	Tyr	Asn	Asp	Pro	Ile	Asp	Asn
1				5				10					15		
Asp	Asn	Ile	Ile	Met	Met	Glu	Pro	Pro	Phe	Ala	Arg	Gly	Thr		
		20					25						30		

&lt;210&gt; 62

<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype B

<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (44)...(44)  
<223> Arginine substitution

<400> 62  
Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys  
1 5 10 15  
Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Tyr Glu Glu  
20 25 30  
Ile Ser Lys Glu His Leu Ala Val Tyr Lys Ile Arg Met Cys Lys Ser  
35 40 45  
Val Lys  
50

<210> 63  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype B

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (21)...(21)  
<223> Alanine substitution

<221> VARIANT  
<222> (22)...(22)  
<223> Alanine substitution

<400> 63  
Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn  
1 5 10 15  
Asp Asn Ile Ile Ala Ala Glu Pro Pro Phe Ala Arg Gly Thr  
20 25 30

<210> 64  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype B

<220>

<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (41)...(41)  
<223> Arginine substitution

<400> 64  
Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys  
1 5 10 15  
Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Tyr Glu Glu  
20 25 30  
Ile Ser Lys Glu His Leu Ala Val Arg Lys Ile Gln Met Cys Lys Ser  
35 40 45  
Val Lys  
50

<210> 65  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype B

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (10)...(10)  
<223> Arginine substitution

<400> 65  
Met Pro Val Thr Ile Asn Asn Phe Asn Arg Asn Asp Pro Ile Asp Asn  
1 5 10 15  
Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr  
20 25 30

<210> 66  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype B

<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (30)...(30)  
<223> Lysine substitution

<400> 66

Tyr Thr Ile Glu Glu Gly Phe Asn Ile Ser Asp Lys Asn Met Gly Lys  
 1 5 10 15  
 Glu Tyr Arg Gly Gln Asn Lys Ala Ile Asn Lys Gln Ala Lys Glu Glu  
 20 25 30  
 Ile Ser Lys Glu His Leu Ala Val Tyr Lys Ile Gln Met Cys Lys Ser  
 35 40 45  
 Val Lys  
 50

<210> 67  
 <211> 30  
 <212> PRT  
 <213> Clostridium botulinum serotype C1

<220>  
 <221> DOMAIN  
 <222> (1)...(30)  
 <223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
 <222> (8)...(8)  
 <223> Lysine substitution

<400> 67  
 Met Pro Ile Thr Ile Asn Asn Lys Asn Tyr Ser Asp Pro Val Asp Asn  
 1 5 10 15  
 Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala  
 20 25 30

<210> 68  
 <211> 50  
 <212> PRT  
 <213> Clostridium botulinum serotype C1

<220>  
 <221> DOMAIN  
 <222> (1)...(50)  
 <223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
 <222> (48)...(48)  
 <223> Arginine substitution

<400> 68  
 Asn Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu  
 1 5 10 15  
 Ser Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr  
 20 25 30  
 Leu Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Arg  
 35 40 45  
 Asn Lys  
 50

<210> 69  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype D

<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain

<221> VARIANT  
<222> (5)...(5)  
<223> Alanine substitution

<221> VARIANT  
<222> (14)...(14)  
<223> Alanine substitution

<400> 69  
Met Thr Trp Pro Ala Lys Asp Phe Asn Tyr Ser Asp Pro Ala Asn Asp  
1 5 10 15  
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile  
20 25 30

<210> 70  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype D

<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain

<221> VARIANT  
<222> (44)...(44)  
<223> Alanine substitution

<400> 70  
Tyr Thr Ile Arg Asp Gly Phe Asn Leu Thr Asn Lys Gly Phe Asn Ile  
1 5 10 15  
Glu Asn Ser Gly Gln Asn Ile Glu Arg Asn Pro Ala Leu Gln Lys Leu  
20 25 30  
Ser Ser Glu Ser Val Val Asp Leu Phe Thr Lys Ala Cys Leu Arg Leu  
35 40 45  
Thr Lys  
50

<210> 71  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype E



&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(30)

&lt;223&gt; Amino terminal 30 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (13)...(13)

&lt;223&gt; Alanine substitution

&lt;400&gt; 71

Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Ala Asn Asp Arg

1 5 10 15

Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr

20 25 30

&lt;210&gt; 72

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype E

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(50)

&lt;223&gt; Carboxyl terminal 50 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (31)...(31)

&lt;223&gt; Histidine substitution

&lt;400&gt; 72

Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe Arg Gly Gln Asn Ala

1 5 10 15

Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr Gly Arg Gly His Val

20 25 30

Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val Ser Val Lys Gly Ile

35 40 45

Arg Lys

50

&lt;210&gt; 73

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype E

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(30)

&lt;223&gt; Amino terminal 30 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (7)...(7)

&lt;223&gt; Arginine substitution

&lt;400&gt; 73

Met	Pro	Lys	Ile	Asn	Ser	Arg	Asn	Tyr	Asn	Asp	Pro	Val	Asn	Asp	Arg
1				5				10					15		
Thr	Ile	Leu	Tyr	Ile	Lys	Pro	Gly	Gly	Cys	Gln	Glu	Phe	Tyr		
			20				25						30		

&lt;210&gt; 74

&lt;211&gt; 50

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype E

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(50)

&lt;223&gt; Carboxyl terminal 50 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (42)...(42)

&lt;223&gt; Alanine substitution

&lt;221&gt; VARIANT

&lt;222&gt; (43)...(43)

&lt;223&gt; Alanine substitution

&lt;400&gt; 74

Gly	Tyr	Asn	Ile	Asn	Asn	Leu	Lys	Val	Asn	Phe	Arg	Gly	Gln	Asn	Ala
1				5				10					15		
Asn	Leu	Asn	Pro	Arg	Ile	Ile	Thr	Pro	Ile	Thr	Gly	Arg	Gly	Leu	Val
			20				25					30			
Lys	Lys	Ile	Ile	Arg	Phe	Cys	Lys	Asn	Ala	Ala	Ser	Val	Lys	Gly	Ile
		35				40					45				
Arg	Lys														
	50														

&lt;210&gt; 75

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum serotype E

&lt;220&gt;

&lt;221&gt; DOMAIN

&lt;222&gt; (1)...(30)

&lt;223&gt; Amino terminal 30 amino acids of light chain

&lt;221&gt; VARIANT

&lt;222&gt; (30)...(30)

&lt;223&gt; Arginine substitution

&lt;400&gt; 75

Met	Pro	Lys	Ile	Asn	Ser	Phe	Asn	Tyr	Asn	Asp	Pro	Val	Asn	Asp	Arg
1				5				10					15		
Thr	Ile	Leu	Tyr	Ile	Lys	Pro	Gly	Gly	Cys	Gln	Glu	Phe	Arg		

20

25

30

<210> 76  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype E  
  
<220>  
<221> DOMAIN  
<222> (1)...(50)  
<223> Carboxyl terminal 50 amino acids of light chain  
  
<221> VARIANT  
<222> (45)...(45)  
<223> Alanine substitution

<400> 76  
Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe Arg Gly Gln Asn Ala  
1 5 10 15  
Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr Gly Arg Gly Leu Val  
20 25 30  
Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val Ser Ala Lys Gly Ile  
35 40 45  
Arg Lys  
50

<210> 77  
<211> 30  
<212> PRT  
<213> Clostridium botulinum serotype F  
  
<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain  
  
<221> VARIANT  
<222> (3)...(3)  
<223> Alanine substitution

<400> 77  
Met Pro Ala Ala Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp  
1 5 10 15  
Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser  
20 25 30

<210> 78  
<211> 50  
<212> PRT  
<213> Clostridium botulinum serotype F  
  
<220>

```
<221> VARIANT
<222> (46)...(46)
<223> Alanine substitution
```

```

<400> 78
Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn Asn Arg
 1          5          10          15
Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile Pro Asp
          20          25          30
Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Ala Ile Pro
          35          40          45
Arg Lys
          50

```

```
<210> 79
<211> 30
<212> PRT
<213> Clostridium botulinum serotype G
```

```
<220>  
<221> DOMAIN  
<222> (1)...(30)  
<223> Amino terminal 30 amino acids of light chain
```

```
<221> VARIANT
<222> (8)...(8)
<223> Histidine substitution
```

```

<400> 79
Met  Pro  Val  Asn  Ile  Lys  Asn  His  Asn  Tyr  Asn  Asp  Pro  Ile  Asn  Asn
  1          5          10          15
Asp  Asp  Ile  Ile  Met  Met  Glu  Pro  Phe  Asn  Asp  Pro  Gly  Pro
          20          25          30

```

```
<210> 80
<211> 50
<212> PRT
<213> Clostridium botulinum serotype G
```

```

<220>
<221> DOMAIN
<222> (1)...(50)
<223> Carboxyl terminal 50 amino acids of light chain

```

```
<221> VARIANT
<222> (47)...(47)
<223> Alanine substitution
```

<400> 80

Gln Asn Glu Gly Phe Asn Ile Ala Ser Lys Asn Leu Lys Thr Glu Phe  
1 5 10 15  
Asn Gly Gln Asn Lys Ala Val Asn Lys Glu Ala Tyr Glu Glu Ile Ser  
20 25 30  
Leu Glu His Leu Val Ile Tyr Arg Ile Ala Met Cys Lys Pro Ala Met  
35 40 45  
Tyr Lys  
50

<210> 81  
<211> 50  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(50)  
<223> Peptide comprising a 6x His tag and S-tag

<400> 81  
Met His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser  
1 5 10 15  
Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp  
20 25 30  
Ser Pro Asp Leu Gly Thr Asp Asp Asp Lys Ala Met Tyr Lys Asp  
35 40 45  
Pro Val  
50

<210> 82  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(14)  
<223> Peptide comprising a 6x His tag

<400> 82  
Asn Phe Thr Lys Leu Thr Arg Ala His His His His His His  
1 5 10

<210> 83  
<211> 59  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> PEPTIDE  
<222> (1)...(59)  
<223> Peptide comprising a 6x His tag and S-tag

&lt;400&gt; 83

Met	His	His	His	His	His	His	Ser	Ser	Gly	Leu	Val	Pro	Arg	Gly	Ser
1				5					10					15	
Gly	Met	Lys	Glu	Thr	Ala	Ala	Ala	Lys	Phe	Glu	Arg	Gln	His	Met	Asp
			20					25					30		
Ser	Pro	Asp	Leu	Gly	Thr	Asp	Asp	Asp	Asp	Lys	Ala	Met	Gly	Ser	Phe
		35					40						45		
Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val					
	50					55									